PATENT COOPERATION TREATY

RECEIVED

MAY 13 2005

From the INTERNATIONAL SEARCHING AUTHORITY

To:
JANE MASSEY LICATA
LICATA & TYRRELL P.C.
66 E. MAIN STREET
MARLTON, NJ 08053

Docket System
Status Report
Docket Book
SR issued 5/11/05
Reply no 8/11/05

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference RCK-0017	Date of mailing *(day/month/year)* 11 MAY 2005 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US04/37925	International filing date *(day/month/year)* 12 November 2004 (12.11.2004)
Applicant THE ROCKEFELLER UNIVERSITY	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 740 14 35

   For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/ US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Thaian N. To Telephone No. 571-272-0500

Form PCT/ISA/220 (January 2004)

(See notes on accompanying sheet)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference RCK-0017	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US04/37925	International filing date (*day/month/year*) 12 November 2004 (12.11.2004)	(Earliest) Priority Date (*day/month/year*) 24 November 2003 (24.11.2003)
Applicant THE ROCKEFELLER UNIVERSITY		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the Report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      ☐ The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. ☐ **Certain claims were found unsearchable** (See Box No. II)
3. ☐ **Unity of invention is lacking** (See Box No. III)
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/37925

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 5/00, 5/02, 15/00, 15/09, 15/63, 15/70, 15/74, 15/85, 15/87; A01K 67/00, 67/03, 67/027
US CL : 435/325, 320.1, 455, 463; 800/13, 14
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/325, 320.1, 455, 463; 800/13, 14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TREMPUS, C.S. et al. Enrichment for Living Murine Keratinocytes from the Hair Follicle Bulge with the Cell Surface Marker CD34. J. Invest. Dermatology. April 2003, Vol 120, No. 4, pages 501-511.	1-6
X	YUAN, X. et al. Expression of the Green Fluorescent Protein in the Oligodendrocyte Lineage: A Transgenic Mouse for Developmental and Physiological Studies. J. of Neurosicence Research. 2002, Vol 70, pages 529-545.	7, 9-16
X	ROY, N.S. et al. Identification, Isolation, and Promoter-Defined Separation of Mitotic Oligodendrocyte Progenitor Cells from the Adult Human Subcortical White Matter. J. of Neuroscience. November 14, 1999, Vol 19, No. 22, pages 9986-9995.	7, 9-16
X	FUJIKAWA, T. et al. Purification of Adult Hepatic Progenitor Cells Using Green Fluorescent Protein (GFP)-Transgenic Mice and Fluorescence-Activated Cell Sorting. J. of Hepatology. 20003, Vol. 39, pages 162-170.	7, 9-16

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
28 April 2005 (28.04.2005)

Date of mailing of the international search report
11 MAY 2005

Name and mailing address of the ISA/US
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Commissioner for Patents
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Thaian N. Ton
Telephone No. 571-272-0500

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/37925

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COFFIN, R.S. et al. Pure Populations of Transduced Primary Human Cells Can Be Produced Using GFP Expressing Herpes Virus Vectors and Flow Cytometry. Gene Therapy. 1998, Vol. 5, pages 718-722.	7, 9-16
X	BARTZ, H. et al. Large-Scale Isolation of Immature Dendritic Cells with Features of Langerhans Cells By Sorting CD34+ Cord Blood Stem Cells Cultured in the Presence of TGF-b1 for Cutaneous Leukocyte Antigen (CLA). J. of Immunological Methods. 2003, Vol. 275, pages 137-148.	7, 8-16
X	PUNZEL, M. et al. The Type of Stromal Feeder Used in Limiting Dilution Assays Influences Frequency and Maintenance of Human Long-Term Culture Initiating Cells. Leukemia. 1999, Vol. 13, page 92-97.	17-18
X	KRESTEL, H.E. et al. A GFP-Equipped Bidirectional Expression Module Well Suited for Monitoring Tetracyline-Regulated Gene Expression in Mouse. Nucleic Acids Research. 2001, Vol. 29, Vo. 7, pages 1-6.	19-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/37925

Continuation of B. FIELDS SEARCHED Item 3:
Caplus, medline, embase, biosis, lifesci, west
Search terms: cell sort, FACS, CD34+, fibroblast, calcium, BMP-6, FGF-18 , hematopoietic, transgenic, tetracycline

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
JANE MASSEY LICATA
LICATA & TYRRELL P.C.
66 E. MAIN STREET
MARLTON, NJ 08053

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	11 MAY 2005
Applicant's or agent's file reference	FOR FURTHER ACTION See paragraph 2 below
RCK-0017	

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/37925	12 November 2004 (12.11.2004)	24 November 2003 (24.11.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12N 5/00, 5/02, 15/00, 15/09, 15/63, 15/70, 15/74, 15/85, 15/87; A01K 67/00, 67/03, 67/027 and US Cl.: 435/325, 320.1, 455, 463; 800/13, 14

Applicant

THE ROCKEFELLER UNIVERSITY

1. This opinion contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US
Mail Stop PCT, Attn: ISA/US
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Authorized officer
Thaian N. Ton
Telephone No. 571-272-0500

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/37925

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ in written format

☐ in computer readable form

c. time of filing/furnishing

☐ contained in international application as filed.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/37925

Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims NONE	YES
	Claims 1-20	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-20	NO
Industrial applicability (IA)	Claims 1-20	YES
	Claims NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/37925

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:
Claims 1-20 the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

Claims 1-6 lack novelty under PCT Article 33(2) as being anticipated by Trempus *et al.* The claims are directed to methods for isolating a self-renewing, multipotent cell by obtaining a cell from a sample and sorting the cells based upon the presence of CD34 and the amount of a selected slow-cycling cell marker expressed by the cell. The claims are also directed to cells isolated by the claimed method. Trempus teach the isolation of epithelial cells with stem and progenitor cell characteristics using a CD34 specific antibody, and identifying in that population a subset of cells also expression alpha-6 integrin. See Abstract. Particularly, they teach that keratinocytes were isolated from the dorsal skin of mice, cells were separated by flow cytometry and the resulting cells isolated. See Materials and Methods, pp. 502-503. Thus, Trempus teach the claimed invention because they teach a progenitor cell isolated by the presence of both CD34 and another marker expressed by the cell.

Claims 7, 9-16 lack novelty under PCT Article 33(2) as being anticipated by Yuan *et al.*, or Roy *et al.*, or Fujikawa *et al.* or Coffin *et al.* Note that claims 9-16 are directed to cell populations, produced by a particular method. The method by which the cells are produced fails to differentiate the cells from the art, thus, art that teaches the products teaches the claims.

Yuan teach the generation of a transgenic mouse expressing EGFP under the CNP promoter. They observe the expression of EGFP, and isolated oligodendrocyte progenitor cells from the mice using fluorescence activated cell-sorting (FACS). See Methods and Materials, p. 530-531.

Roy teach the identification isolation of oligodendrocyte progenitor cells from adult human subcortical white matter. Particularly, they teach the dissociation and culture of cells from adult human brain (p. 9987, Materials and Methods, 2nd column), the transfection of these cells with a transgene eoncding the CNP2 promoter with targeted GFP expression. They teach that the cells expressing GFP were then sorted using flow cytometry and a FACS machine. See p. 9989, 1st column.

Fujikawa teach the purification of isolated hepatic progenitor cells using GFP-transgenic mice, and isolating cells from the mice. Paritcularly, they teach that GFP-transgenic mice, which express GFP under the cytomegalovirus enhancer-beta-actin promoter. Liver tissues were isolated from the mice, and then the cells were sorted and characterized. The cells were then sorted by FACS and analyzed. See pp. 163-164. Fujikawa teach that the cells that were sorted had immature characteristics (p. 166, 2nd column) and that the cells showed *in vitro* differentiation potential to produce hepatocytes. See p. 167, #3.5.

Coffin teach the generation of populations of transduced human primary cells by FACS sorting using GFP expression. Particularly, they teach that human hematopoietic stem cells were transduced using a HSV1 vector expressing GFP. See Abstract. The transduced cells were then sorted to remove GFP-negative cells.

Claims 7, 8-16 lack novelty under PCT Article 33(2) as being anticipated by Bartz *et al.* Bartz teach the isolation of immature dendritic cells from Langerhans cells by sorting using two markers, CD34+ or CD133+ (see p. 139, #2.3) and then cells from this population were further sorted and isolated using CLA expression (p. 139, #2.4). The resulting cells were the isolated and cultured and then analyzed (p. 139, #2.5).

International application No.
PCT/US04/37925

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

Claims 17-18 lack novelty under PCT Article 33(2) as being anticipated by Punzel *et al.* Puzel teach the culture and expansion of human hematopoietic stem cells, by growing the cells on fibroblast feeder cells using LTBMC medium. See p. 93, 2nd column. Note that the LTBMC medium that they teach contains IMDM, which contains calcium chloride (.219 g/L). Thus, they anticipate the claims.

Claims 19-20 lack novelty under PCT Article 33(2) as being anticipated by Krestel *et al.* Krestel teach the generation of transgenic mice using a transgene encoding humanized GFP that is regulated by doxycycline. Expression was activated when the transcription factor tTA (tet-dependent transcirpiton activator) was expressed by the transgene. See Abstract and Materials and Methods.